Oxitec, Ltd. Submission to Docket EPA-HQ-OPP-2015-0374

Oxitec, Ltd., hereby submits comments on EPA's April 26, 2016 Notice announcing receipt of application 88877-EUP-2, submitted by the University of Kentucky Department of Entomology (UKDE) requesting an amendment and extension to an existing experimental use permit (EUP) for *Wolbachia pipientis*, strain wAlbB. Oxitec submits these comments to strongly convey that prior to approval of the uncontained release to the environment of substantial numbers of modified mosquitoes carrying this strain of *Wolbachia pipientis* EPA must conduct a sufficiently rigorous assessment of the potential adverse impacts to human health and the environment that such uncontained release of modified mosquitoes may entail. As we discuss in detail below, Oxitec believes that there are significant questions regarding these potential impacts and these must be addressed prior to any regulatory approval.

Moreover, it is not clear from the public record of this matter if sufficient data and information have been submitted by the applicant to address these issues. The information available in public docket EPA-HQ-OPP-2015-0374 for the *Wolbachia* EUP application 88877-EUP-2 is extremely limited. The paucity of information available in the docket makes it difficult for the public to comment on whether the application should be granted, or what should be the scope of any potential approval of the EUP. For the public in general, and Oxitec in particular, to provide meaningful comment on whether this application to amend and extend the EUP should be granted, more information relevant to a determination of environmental and human safety should be available as part of the public record. Moreover, should EPA determine after review to grant the application for amendment and extension of the EUP, EPA must make available all data and information supporting such decision, and clearly explain how the critical human health and ecological issues raised in these comments are addressed.

At the outset, we note that Oxitec has developed a different genetic insect control technology that has been demonstrated to be efficacious in significantly reducing the population of disease-carrying mosquitoes (>90% in the Cayman Islands, Brazil, and Panama). Because Oxitec's insect control technology utilises genetic engineering of the insect genome, it has been determined that it is to be regulated by the U.S. Food and Drug Administration as a new animal drug. It is the oft-stated policy of the U.S. government that it regulates the products of biotechnology on the basis of a "risk-based, scientifically sound approach . . . that focuses on the characteristics of the biotechnology product and the environment into which it is being introduced, not the process by which the product is created." *Exercise of Federal Oversight Within Scope of Statutory Authority; Planned Introductions of Biotechnology Products Into the Environment*, 57 Fed. Reg. 6753 (Feb. 27, 1992) (this seminal statement is repeated throughout the Policy Statement, see, e.g., *Id.* at 6754-55, 6755, 6756, 6757, and 6760). Notwithstanding this consistently stated position, the reality is that Oxitec's self-limiting mosquitoes have been subjected to a mandatory pre-market approval regulatory process at FDA that has been much more onerous than the regulatory requirements faced by the *Wolbachia pipientis* microbial pesticide at EPA. The distinction between the EPA review process for *Wolbachia*, and that faced by Oxitec's self-limiting mosquitoes has even been noted by *Nature* [1].

As is detailed below, horizontal gene transfer could result in *Wolbachia* effectively introducing over one thousand new genes into the recipient organism. Notwithstanding this potential for indiscriminate gene transfer, the *Wolbachia* IIT (Incompatible Insect Technique) vector control method is subjected to a substantially less rigorous regulatory review process than is a targeted genetic engineering methodology. This is directly contrary to the intent of the Coordinated Framework and to a scientifically valid risk-based regulatory process.

Such a disparate regulatory approach to two products intent on achieving similar public health ends is inconsistent with the stated Federal regulatory policy, and, as a matter of risk-based regulatory process, is without reason and justification. Oxitec's self-limiting technology and the *Wolbachia* IIT approach both have the intended purpose of end point reductions in the population of mosquitoes and involve releases of substantial numbers of non-wild type mosquitoes to the environment. Importantly, however, Oxitec's self-limiting genetic engineering is well-defined and includes only two well-studied genes, which were purposefully added and reviewed by the regulatory agencies, whereas the potential genetic modification that may result from use of the wAlbB *Wolbachia* bacterium is wholly undefined. Several studies have shown that horizontal gene transfer between *Wolbachia* and their insect hosts may result in gene transfers ranging from nearly the entire *Wolbachia* genome (>1 megabase) to short insertions (<500 base pairs) into various hosts [2-5]. Effectively the *Wolbachia* IIT approach could introduce over one thousand new genes into the target mosquito with unknown consequences, and if they provide a positive selection to the mosquito in the environment this could result in novel strains pervading and spreading through the population. Therefore, as a matter of sound regulatory action, it is incongruous for the regulatory burden placed on the *Wolbachia* IIT technology to be significantly less onerous and burdensome than the requirements imposed on Oxitec's self-limiting targeted genetic engineering technology.

As an example of the disparate regulatory treatment that Oxitec's self-limiting mosquitoes have faced, FDA's Center for Veterinary Medicine (CVM) established an Animal Biotechnology Interdisciplinary Group (ABIG) to evaluate Oxitec's technology. This ABIG included experts from FDA/CVM, CDC, and EPA. It is not clear from the record if the UKDE EUP application was shared with other regulatory agencies and if they were given a chance to comment. In this regard, we believe that the FDA review process for Oxitec's self-limiting technology raised questions and considerations that are relevant to application 88877-EUP-2 to amend and extend the current *Wolbachia* EUP.

Thus, consistent with the regulatory questions that Oxitec has had to address, it is critical to know whether the following questions will be addressed for the *Wolbachia* mosquitoes:

- Can the *Wolbachia* mosquito escape the confined conditions in which it is reared?
- What is the likelihood that the *Wolbachia* mosquitoes will survive and disperse once released into the environment?
- What is the likelihood that *Wolbachia* mosquitoes can reproduce and establish in the environment into which they are released?
- What are the potential impacts of the *Wolbachia* mosquitoes in the environment, including on humans?

- What are the likely consequences for the surrounding environment, should the *Wolbachia* mosquitoes survive and establish in the environment?

In addition, to properly comment on whether the UKDE EUP application should be granted, the answers to basic ecological risk assessment questions must be known, as well as the basis for such answers. We highlight below specific questions, risk considerations, and scientific data and information relevant to the ecological and human health risk assessment of release of *Wolbachia* mosquitoes that must be addressed in this action.

What is the likelihood that Wolbachia mosquitoes can reproduce and establish in the environment into which they are released?

- *Wolbachia* is a bacterium residing within the cells of insects, and is passed through vertical transmission from mother to offspring. Even a single *Wolbachia* infected female could lay hundreds of eggs that would invade the wild population, rendering the Incompatible Insect Technique ineffective and spreading a new strain of *Wolbachia* into the environment. Modelling has shown that conditions of lower competition can favour infected females [6-8]. In other words, as a mosquito population is reduced, or if a population is already low, the chances of *Wolbachia* invading the wild population are increased.

- Given that the release of females carrying the *Wolbachia* strain in the cytoplasm is to be avoided, sex sorting should approach 100%. Yet, studies have shown that mechanical sex sorting with IIT is only 90% effective in removing females. Thus, the reported inefficiency of sex sorting of *Aedes aegypti* mosquitoes infected with *Wolbachia* presents a risk that must be evaluated [9].

- Any unexpected effects of *Wolbachia* could persist in the wild if any females are released, with little possibility of recall [10].

- According to the proposed EUP label: "Only male (and no female) *Ae. aegypti* strain WB1 mosquitoes carrying *Wolbachia pipientis*, wAlbB Strain microbial pesticide are to be released." We question whether this statement is correct. No information has been publicly provided to demonstrate that the sorting methods used to generate populations of mosquitoes to be released guarantee that females will not be released. Nor has information been provided regarding quality control monitoring that will be conducted to ensure this.

- The permit requires the user to 'sample released mosquitoes to confirm the rate of female mosquitoes released'. But nowhere does it say how this will be done. As this is such an important aspect of the safety and efficacy of the system any EUP that allows release of *Wolbachia* mosquitoes should be revoked and releases stopped if females are detected.

What are the potential impacts of the mosquitoes in the environment, including on humans?

- *Aedes aegypti* is not naturally infected with any *Wolbachia* strain, therefore this is an invasive infection in this species. The *Wolbachia* is an endosymbiont on the cytoplasm of the cell so over a thousand new genes are introduced into the insect cells, some or all of which have the potential to randomly integrate into the insect's nuclear genome with unknown consequences. Moreover, the possible persistence of *Wolbachia* mosquitoes themselves is a significant concern. For the reasons set forth below, each new strain of mosquito, or indeed any artificially *Wolbachia* infected insect needs to be treated as a new strain and thoroughly tested in the laboratory before any field releases.

    - *Aedes aegypti* artificially modified with *Wolbachia* show a reduction in dengue virus replication but virus is still found in the saliva of these engineered mosquitoes which therefore have the capacity, even if reduced, to transmit disease [11].
    - The whole genome of *Wolbachia* can transfer to a host genome, meaning a host mosquito could be transformed with over one thousand new genes with unpredictable results [2-5].
    - It has already been shown that horizontal gene transfer (HGT) can transfer genes between *Wolbachia* and its host in *Aedes aegypti* [12] and several other mosquito species [13]. Therefore, *Wolbachia* can genetically transform its host with functional genes with currently unknown consequences.
    - Widespread recombination occurs throughout the *Wolbachia* genome [14], increasing the likelihood of genes changing as the *Wolbachia* evolves. In addition *Wolbachia* has been shown to change its phenotypic effects on the host insect as it evolves [15]. This could potentially change how *Wolbachia* responds to a number of factors, including how it influences host immune response and vectorial status. Therefore, potentially the vectorial capacity of *Aedes aegypti* infected with *Wolbachia* could change over time and should be continually assessed.
    - Research has shown the mosquito's microbiome can impede vertical transmission of *Wolbachia* [16].
    - Temperature impacts *Wolbachia*-malaria interaction in mosquitoes suggesting impact of transfection might vary across diverse environments [17].
    - Research has shown *Wolbachia* enhances West Nile virus infection in the mosquito *Culex tarsalis*. This introduces the possibility that the *Wolbachia* infection could spread to *Culex* populations in areas where West Nile virus is a concern [18].
    - Research has shown *Wolbachia* can enhance malaria parasite infection in two genera of mosquitoes [19-21].

- Further work is needed to define the underlying molecular mechanisms of *Wolbachia* induced reproductive modifications, particularly cytoplasmic incompatibility [22].

- Based on the available record, it is not clear what level of environmental assessment has been conducted to this point. Because *Wolbachia* has been demonstrated to affect insects in the environment – changing their behaviour, disease transmission status, gene expression and biology [6, 23, 24] – Oxitec believes that approval of widespread release of this modified organism without a comprehensive ecological risk assessment is wholly inconsistent with EPA's statutory and regulatory responsibilities under FIFRA.

- Moreover, it is not unreasonable to surmise that the pathogen may evolve in response to *Wolbachia* infection in mosquitoes, with potential adverse results. Oxitec believes that additional information and analysis is required regarding interactions of host insect, the pathogen, and *Wolbachia*, to ensure that *Wolbachia* does not ultimately select for a more dangerous pathogen [22].

- Horizontal transmission between unrelated host species is a proven phenomenon in *Wolbachia* [25]. Studies have demonstrated that genetic sequences, ranging in size from single genes to entire bacterial genomes, have been transferred from *Wolbachia* to many of their insect hosts [2-5], and its effect on disease transmission is variable and potentially dangerous. We note that the Florida Keys has many national parks and protected species that could be susceptible to infection with *Wolbachia* with unknown consequences on the ecosystem. This is another reason that a full-fledged ecological risk assessment is appropriate prior to approval of the requested amendment and extension of the EUP.

- There is evidence that male age and overcrowding during development (*i.e.*, under mass rearing conditions required to produce enough males for IIT to be effective) can reduce the cytoplasmic incompatibility effect in certain insects, rendering the males fertile [26] and able to spread the *Wolbachia* infection through surviving females. Has this study been performed on *Aedes aegypti* to look at age effects and rearing conditions on the cytoplasmic incompatibility penetrance? If not, this should be performed as this is another potential route to simply spread a new *Wolbachia* species into the Florida Keys environment with unknown consequences.

- The release of any females as a result of mis-sorting for any reason would allow the *Wolbachia* strain to invade the wild populations of mosquitoes in the release area, thus rendering this control approach ineffective and with the potential to spread a new strain of *Wolbachia* into the environment with unknown outcomes and consequences.

- Introduction of *Wolbachia* into a mosquito provides the possibility to introduce over time over one thousand new genes, yet *Wolbachia* mosquitoes have not been subjected to the rigorous regulatory scrutiny that appears to be the norm for recombinant genetic modification (notwithstanding that typical genetic modification is a 'rifle shot' approach, involving very few, fully characterised, introduced genes). As noted above, this seems entirely incongruent with the stated policy of the U.S. government as set forth in the Coordinated Framework. How can it be ensured that introduction of genes of unknown

function are not transferred into the *Aedes aegypti* genome and how will this be tested and monitored through time?

In conclusion, Oxitec requests that EPA make publicly available for comment the data and information on which it bases its regulatory decision on the UKDE EUP application, and explain the basis for that decision. Moreover, consistent with FIFRA Section 5(d), EPA should, prior to this strain of *Wolbachia pipientis* being considered for registration and consistent with the serious concerns identified above, specify to the applicant specific data and information that must be provided to address the ecological and human health concerns identified in these comments regarding unreasonable adverse effects on the environment. Consistent with EPA's FIFRA public transparency policy, EPA should allow a public comment period of at least 30 days prior to any approval of the EUP application 88877-EUP-2.

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